Exhibit A

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

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1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

If signal is still seen after autoradiography, rewash using harsher conditions.

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.

REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5× SSC (*APPENDIX 2*)
5× Denhardt solution (*APPENDIX 2*)
1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at −20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

Formamide prehybridization/hybridization (FPH) solution

5× SSC (*APPENDIX 2*)
5× Denhardt solution (*APPENDIX 2*)
50% (w/v) formamide
1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.

CAUTION: *Formamide is a teratogen. Handle with care.*

Labeling buffer

200 mM Tris·Cl, pH 7.5
30 mM $MgCl_2$
10 mM spermidine

Mild stripping solution

5 mM Tris·Cl, pH 8.0
2 mM EDTA
0.1× Denhardt solution (*APPENDIX 2*)

Exhibit B

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SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
H_2O to 1000 ml
Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 µg/ml bovine serum albumin (Pentax Fraction V)
10 mM 2-mercaptoethanol
Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ in 800 ml H_2O
Add H_2O to 1 liter
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2·3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4·H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4·7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate·$2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:	*Working solution, pH ~8.5:*
242 g Tris base	
57.1 ml glacial acetic acid	40 mM Tris·acetate
37.2 g $Na_2EDTA·2H_2O$	2 mM $Na_2EDTA·2H_2O$
H_2O to 1 liter	

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (20 mM)